
CERTIFICATE OF MAILING - EXPRESS MAIL

"Express Mail" mailing label number ET 022 828 332 US
Date of Deposit October 8, 2001

1626-1116

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Director of Patents, United States Patent and Trademark Office, Washington, D.C. 20231.

Mitchell P. Novick

MITCHELL P. NOVICK

**Application for Letters Patent
of the United States**

INVENTORS: MICHAEL B. DANCU
JOHN M. TARBELL

**TITLE OF
INVENTION:** SYSTEM AND METHOD TO SIMULATE
HEMODYNAMICS

Attorneys: Mitchell P. Novick, Esq.
LAW OFFICES OF MITCHELL P. NOVICK
66 Park Street
Montclair, New Jersey 07042
(973) 744-5150

1 SYSTEM AND METHOD TO SIMULATE HEMODYNAMICS
2
3

4 CROSS-REFERENCE TO RELATED APPLICATIONS
5
6

7 This application claims the benefit, under 35 U.S.C. §119(e), of U.S. Provisional
8 Application No. 60/239,015, filed 6 October 2000 by the applicant, and which is herein
9 incorporated by reference in its entirety.
10
11

12 STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT.
13
14

15 The work described in this application was supported by funding from the
16 National Institutes of Health under Grant No. HL-35549. The United States Government may
17 have certain rights to the invention.
18
19

20 FIELD OF THE INVENTION.
21
22

23 The present invention is a system and method for simulating the hemodynamic
24 patterns of physiologic blood flow. In particular, the present invention can simultaneously
25 generate wall shear stress and circumferential strain patterns relevant to cardiovascular
26 function and disease.
27
28

29 BACKGROUND OF THE INVENTION.
30
31

32 Cardiovascular disease is the leading cause of death in the United States, and
33 costs millions of dollars per year. Atherosclerosis is the leading cause of death in the
34 developed world and nearly the leading cause in the developing world. Atherosclerosis is a
35 disorder in which the coronary arteries become clogged by the build up of plaque along the
36 interior walls of the arteries, leading to decreased blood flow which can in turn cause
hypertension, ischemias, strokes and, potentially, death.

Atherosclerosis has been shown to occur in sites of complex hemodynamic
behavior. Surgical intervention is often employed to treat it, and may include insertion of a

1 balloon catheter to clean out the plaque, and insertion of a stent within the vessel to enable
2 it to remain open, or may include multiple bypasses of the clogged vessels. Bypass surgery
3 involves the removal of a section of vein from the patient's lower leg, and its transplant into
4 the appropriate cardiac blood vessels so that blood flows through the transplanted vein and
5 thus bypasses the clogged vessels.

6
7 A major problem associated with bypass surgery is the patency of the vessels
8 to be used in the bypass. The bypass vessels are prone to failure, which may occur within a
9 short period of time after bypass surgery, or after a period of several years. Hemodynamic
10 forces have been implicated as a major factor contributing to the failure of the bypass vessels.

11
12
13 Hemodynamic forces (i.e., forces due to blood flow) are known to influence
14 blood vessel structure and pathology. The vascular cells lining all blood vessels are
15 endothelial cells, which are important sensors and transducers of the two major hemodynamic
16 forces to which they are exposed: wall shear stress ("WSS"), which is the fluid frictional force
17 per unit of surface area, and hoop stress, which is driven by the circumferential strain ("CS")
18 of pressure changes. Wall shear stress acts along the blood vessel's longitudinal axis.
19 Circumferential strain is associated with the deformation of the elastic artery wall (i.e.,
20 changes in the diameter of the vessel) in response to the pulse of vascular pressure. Wave
21 reflections in the circulation and the inertial effects of blood flow cause a phase difference,
22 the stress phase angle ("SPA"), between CS and WSS. The SPA varies significantly throughout
23 the circulation, and is most negative in disease prone locations, such as the outer walls of a
24 blood vessel bifurcation. Hemodynamic forces have been shown to dramatically alter
25 endothelial cell function and phenotype (i.e., high shear stress [low SPA] is associated with
26 an atheroprotective gene expression profile, and a low shear stress [large SPA] is associated
27 with an atherogenic gene expression profile). There is thus a great need to study vascular
28 biology in a complete, integrative, and controlled hemodynamic environment.

29
30 Despite the significance of hemodynamic WSS and CS acting on the vessel wall,
31 especially at regions of the circulation with a high risk of localization of cardiovascular
32 diseases, detailed knowledge of the combined influence of the time varying patterns of WSS
33 and CS on endothelial cell biological response has remained technologically unfeasible.

34
35 Laboratory studies of vascular fluid mechanics have demonstrated that wall
36 shear stress (WSS) and circumferential strain (CS) are out of phase temporally, and that there

1 is a systematic variation of the stress phase angle (SPA) throughout the circulation. This
2 variation is highly out-of-phase in the large arteries, where arterial disease generally occurs,
3 while in the smaller vessels and veins where disease is rare, this variation is generally in-
4 phase.

5
6 Where an artery bifurcates, SPA varies with the local spatial position within that
7 bifurcation, the more out-of-phase environment being localized on the outer wall of the
8 bifurcation where atherosclerosis occurs. SPA was found to be more out-of-phase in the
9 coronary arteries than at any other location in the circulation.

10
11 Prior technology has focused on the individual effects of WSS or CS,
12 individually, on endothelial cells. Berthiaume and Frangos described a device that simulates
13 wall shear stress using a rod and plate system that is similar to the cone and plate system
14 used in viscometers. Chang described a parallel flow chamber used to simulate steady flow.
15 Carosi et al and Sumpio et al, describe devices to simulate cyclic strain that consists of a
16 flexible membrane that is stretched by a motor or a vacuum suction system.

17
18 Qiu and Tarbell described a device to simulate pressure and flow in tubes, but
19 the device did not permit using a wide range of phase angles (SPAs), and was technically
20 difficult to use. Limitations, however, of the Qiu and Tarbell system included having the
21 maximum attainable phase angle being 100 degrees, the amplitude and phase of the flow and
22 pressure are coupled, and the system utilized large quantities of fluid. The present invention,
23 by its selection of tubing and vessel diameters, in contrast, employs approximately one fifth
24 the volume of fluid as that system. Seliktar et al., in an in vitro study, verified that simulation
25 of the hemodynamic environment is critical to vessel patency and function.

26
27 The patent literature described several systems for examining the effects of
28 strain, or the effects of shear, individually, on cells or blood vessels.

29
30 Seliktar et al. (U.S. Pat. No. 5,928,945) describes a bioreactor for producing
31 cartilage in vitro, comprising a growth chamber, a substrate on which chondrocyte cells or
32 chondrocyte stem cells are attached, and means for applying relative movement between a
33 liquid culture medium and the substrate to provide a shear flow stress to the cells attached
34 to the substrate.

35
36 In U.S. Pat. No. 5,899,937 Goldstein et al. describe a closed, sterile pulsatile

1 loop for studying tissue valves. The system provides a tool to examine heart valve leaflet
2 fibroblast function and differentiation as these are affected by mechanical loading, as well as
3 an apparatus to provide heart valves seeded with suitable cells. The sterile pulsatile flow
4 system which exposes viable tissue valves to a dynamic flow environment imitating that of the
5 aortic valve.

6

7 Wolf et al. (U.S. Pat. No. 5,271,898) discloses an apparatus for testing
8 blood/biomaterials/device interactions and characteristics, comprising a stepper-motor driven
9 circular disc upon which a test vehicle is mounted. The test vehicle comprises a circular,
10 closed loop of polymer tubing containing a check valve, and contains either the test materials,
11 coating, or device. The apparatus generates pulsatile movement of the test vehicle.
12 Oscillation of the test vehicle results in the pulsatile movement of fluid over its surface.

13

14 In U.S. Pat. No. 6,205,871 B1 Saloner et al disclose a panel of anatomically
15 accurate vascular phantoms comprising a range of stenotic conditions varying from normal
16 to critically stenosed (0% area reduction to greater than 99% reduction), and which phantoms
17 are subjected to pulsatile flow of a blood mimic fluid.

18

19 Vilendrer (U.S. Pat. No. 5,670,708) discloses a device for measuring compliance
20 conditions of a prosthesis under simulated physiologic loading conditions. The prosthesis
21 includes stents, grafts and stent-grafts, which is positioned within a fluid conduit of the
22 apparatus, wherein the fluid conduit is filled with a saline solution or other fluid
23 approximating the physiological condition to be tested. The fluids are forced through the fluid
24 conduit from both ends of the conduit in a pulsating fashion at a high frequency simulating
25 systolic and diastolic pressures.

26

27 In U.S. Pat. No. 4,839,280 Banes describes an apparatus for applying stress to
28 cell cultures, comprising at least one cell culture plate having one or more wells thereon, with
29 each of the wells having a substantially planar base formed at least partially of an elastomeric
30 membrane made of biocompatible polyorganosiloxane composition, with the elastomeric
31 membrane having an upper surface treated to permit cell growth and attachment thereto by
32 means of the incorporation at the upper surface of a substance selected from the group
33 consisting of an amine, a carboxylic acid, or elemental carbon, and vacuum means for
34 controlling the elastomeric membrane to the pulling force of a vacuum. Banes (U.S. Pat. No.
35 6,218,178 B1) discloses an improvement, in the form of a loading station assembly for
36 allowing stretching of a flexible cell culture membrane, the assembly comprising a planar

1 member and a post extending from a surface of the planar member, an upper surface of the
2 post being configured to support a flexible cell culture membrane, the planar member defining
3 a passageway configured to allow fluid to flow through from one side of the planar member
4 to an opposite side of the planar member, and wherein the flexible cell culture member is
5 stretchable at a periphery of the upper surface towards the planar member.

6
7 In U.S. Pat. Nos. 4,940,853 and 5,153,136 Vanderburgh describes a method and
8 apparatus for growing tissue culture specimens in vitro, respectively. The apparatus
9 comprises an expandable membrane for receiving a tissue specimen thereon, a mechanism
10 for expanding the membrane and the tissue specimen, and a controller for controlling the
11 expanding mechanism. The controller is operative for applying an activity pattern to the
12 membrane and a tissue specimen thereon which includes simultaneous continuous stretch
13 activity and repetitive stretch and release activity. The continuous stretch and release activity
14 simulate the types of activity to which cells are exposed in vivo due to growth and movement,
15 respectively, and they cause the cells of tissue specimens grown in the apparatus to develop
16 as three-dimensional structures similar to those grown in vivo.

17
18 In U.S. Pat. Nos. 5,217,899 and 5,348,879 Shapiro et al. describe an apparatus
19 and method for stretching cells in vitro, respectively. The inventions impart to a living culture
20 of cells biaxial mechanical forces which approximate the mechanical forces to which cells are
21 subjected in vivo. The apparatus includes a displacement applicator which may be actuated
22 to contact and stretch a membrane having a living cell culture mounted thereon. Stretching
23 of the membrane imparts biaxial mechanical forces to the cells. These forces may be
24 uniformly applied to the cells, or they may be selectively non-uniformly applied.

25
26 Lee et al. (U.S. Pat. No. 6,057,150) discloses a biaxial strain system for cultured
27 cells that includes a support with an opening over which an elastic membrane is secured, a
28 moveable cylinder coaxial with the opening and fitting closely but movably within the opening,
29 and an actuating member that stabilizes and controls the position of the cylinder relative to
30 the opening. The actuating member is coupled to the support by a threaded connection while
31 engaging the movable cylinder. The degree of membrane stretch is accurately controlled by
32 the rotation of the actuating member.

33
34 In U.S. Pat. No. 4,851,354 Winston et al. disclose an apparatus for mechanically
35 stimulating cells, comprising an airtight well having an optically transparent compliant base
36 of a biologically compatible material on which the cells may be grown and an optically

1 transparent, removable cap, coupled with a ported, airtight reservoir which reservoir has an
2 optically transparent base and which reservoir can be filled with pressuring media to create
3 cyclic variations in hydrostatic pressure beneath the compliant base, causing the compliant
4 base to deform and thereby exert a substantially uniform biaxial force on the cells attached
5 thereto.

6
7 Lintilhac et al. (U.S. Pat. No. 5,406,853) disclose an instrument for the
8 application of controlled mechanical loads to tissues in sterile culture. A slider which contacts
9 the test subject is in force transmitting relation to a forcing frame. Tension, compressive and
10 bending forces can be applied to the test subject, and force applied to the test subject is
11 measured and controlled. A dimensional characteristic of the test subject, such as growth,
12 is measured by a linear variable differential transformer. The growth measurement data can
13 be used to control the force applied. Substantially biaxial stretching is achieved by placing
14 the test subject on an elastic membrane stretched by an arrangement of members securing
15 the elastic member to the forcing frame.
16

17 In U.S. Pat. No. 6,107,081 Feeback et al. disclose a uni-directional cell
18 stretching device capable of mimicking linear tissue loading profiles, comprising a tissue
19 culture vessel, an actuator assembly having a relatively fixed structure and an axially
20 transformable ram within the vessel, at least one elastic strip which is coated with an
21 extracellular matrix, and a driving means for axially translating the ram relative to the
22 relatively fixed structure, and for axially translating the end portion of the elastic strap affixed
23 to the ram relative to another, opposite end portion, for longitudinally stretching the elastic
24 strap.
25

26 Nguyen et al. (U.S. Pat. No. 5,272,909) disclose a method and device for testing
27 venous valves in vitro. The device comprises (a) a fixture for mounting a sample valve on a
28 liquid flow path. (b) a muscle pump component and/or (c) respiratory pump component and/or
29 (d) capacitance reservoir component and/or (e) vertical hydrostatic column component, all of
30 the components being fluidly connected to the flow path to mimic the muscle pump,
31 respiratory pump, capacitance and hydrostatic impedance effects of actual in situ venous
32 circulation in the mammalian body. The muscle pump is designed to mimic effects caused by
33 movement of the visceral organs and somatic muscles on a vein, while the respiratory pump
34 is designed to mimic the effects of normal cyclic variations in the intra-thoracic pressure due to
35 the movement of the thoracic muscles and diaphragm. The combination of pumps of the
36 present invention provides a means to examine the effects of pulsatile pressure, wall shear

1 stress, and circumferential strain, separately or in combination, on blood vessels or
2 mammalian cells in vitro.

3
4 In U.S. Pat. No. 5,537,335 Antaki et al. disclose a fluid delivery apparatus in
5 which a predetermined pressure waveform is introduced into a conduit, such as a human
6 saphenous vein. By such exposure, the vein can be "arterialized", meaning that it can be
7 conditioned in preparation for its use in bypass surgery. an excised vein according to the
8 inventors. The combination of pumps and the manner of controlling the degree of their being
9 in phase or out-of-phase with each other provides a means to examine not only the effects of
10 a blood pressure waveform, but also the effects of pulsatile pressure, wall shear stress, and
11 circumferential strain, separately or in combination, on blood vessels or mammalian cells in
12 vitro.
13

14 The most common WSS simulating systems utilize a 2-dimensional stiff surface,
15 such as a glass slide, for the endothelial cell culture forming the wall of a parallel plate flow
16 chamber. The WSS in these devices is usually steady because of difficulties in simulating
17 pulsatile flow. Cyclic straining devices provide only strain, by stretching cells on a compliant
18 membrane without flow. Both types of systems are thus limited by their design. However,
19 no studies have been performed studying both parameters (WSS and CS) using cells grown
20 on a single type of support surface because such a system, until now, has remained
21 technologically unfeasible. The present invention addresses and solves this long-felt need by
22 providing a system in which endothelial cells can be grown on a single support surface, and
23 subjected to studies in which both wall shear stress and circumferential stress can be
24 examined independently of each other.
25

26 The use of a silicone tube coated with endothelial cells was recently introduced,
27 and provided the potential for simultaneous coupled pulsatile strain and shear stress.
28 However, these tubes were used in flow simulators coupling pressure and flow that could only
29 achieve phase angles (SPAs) of about 90-100 degrees; such a phase angle was inadequate for
30 simulating coronary arteries, the most disease prone vessels in the circulation, because
31 coronary arteries are characterized by a high SPA, on the order of approximately 250 degrees.
32 These flow simulators were difficult to use and to produce replicable reliable results. The
33 present invention overcomes this problem, by providing time-varying uniform cyclic pressure
34 (and consequently CS) and pulsatile flow (and consequently WSS) in a 3-dimensional
35 configuration over a complete range of SPAs, as a most complete physiologic environment.
36

1 BRIEF SUMMARY OF THE INVENTION.

2

3 It is an object of the present invention to provide a system to simulate
4 physiological hemodynamics.

5

6 Another object of the present invention to provide a system to simulate
7 biomechanical stimuli due to fluid flow, pressure and pressure differentials (transmural
8 pressure).

9

10 Another object of the present invention is to provide a system in which the
11 effects of wall shear stress ("WSS") and circumferential strain ("CS") can be studied
12 independently of each other.

13

14 Another object of the present invention is to provide a system in which the
15 effects of wall shear stress ("WSS") and circumferential strain ("CS") can be studied
16 simultaneously.

17

18 Another object of the present invention is to provide a system in which the
19 effects of wall shear stress ("WSS") and circumferential strain ("CS") can be studied
20 independently of each other over a wide range of stress phase angles ("SPA").

21

22 Another object of the present invention is to provide a system in which the
23 effects of vasoactive compounds can be studied.

24

25 Another object of the present invention is to provide a system in which effects
26 of vasoactive compounds can be studied on the genes that regulate their production.

27

28 It is an object of the present invention to provide a system to simulate
29 physiological hemodynamics of a plurality of blood vessels.

30

31 It is an object of the present invention to provide a system to simulate
32 physiological hemodynamics of a plurality of mammalian blood vessels.

33

34 It is an object of the present invention to provide a system to simulate
35 physiological hemodynamics of a plurality of human blood vessels.

1 It is an object of the present invention to provide a method for simulating
2 physiological hemodynamics.

3
4 Another object of the present invention to provide a method of simulating
5 biomechanical stimuli due to fluid flow, pressure and pressure differentials (transmural
6 pressure).

7
8 Another object of the present invention is to provide a method for studying
9 effects of wall shear stress ("WSS") and circumferential strain ("CS") independently of each
10 other.

11
12 Another object of the present invention is to provide a method for the
13 simultaneous study of the effects of wall shear stress ("WSS") and circumferential strain
14 ("CS") on vessels.

15
16 Another object of the present invention is to provide a method for the
17 independent study of the effects of wall shear stress ("WSS") and circumferential strain ("CS")
18 over a wide range of stress phase angles ("SPA").

19 Another object of the present invention is to provide a method for studying the
20 effects of vasoactive compounds.

21
22 Another object of the present invention is to provide a method for studying the
23 effects of vasoactive compounds on the genes that regulate their production.

24
25 It is an object of the present invention to provide a method for simulating
26 physiological hemodynamics of a plurality of blood vessels.

27
28 It is an object of the present invention to provide a method for simulating
29 physiological hemodynamics of a plurality of mammalian blood vessels.

30
31 It is an object of the present invention to provide a method for simulating
32 physiological hemodynamics of a plurality of human blood vessels.

33
34 The present invention achieves the uncoupling of pulsatile flow and pulsatile
35 pressure to provide independent control over WSS and CS. The system at first seems
36 paradoxical since it is classically well known that pressure and flow are coupled. However,

1 in a dynamic sinusoidal environment, such as that of the present invention, flow and pressure
2 can be independently modulated and therefore, appear to be uncoupled. The drive system,
3 comprising two reciprocating drive shafts that are coupled via a circular cam effects this
4 uncoupling. The flow shaft drives pumps, that are at opposite ends, that are 180 degrees out-
5 of-phase and are connected to the recirculating flow loop upstream and downstream of the
6 test section (compliant vessel). The flow shaft allows independent control of pulsatile flow
7 with no pulsatile circumferential strain. The second (pressure) shaft also drives two piston
8 pumps that are 180 degrees out-of-phase; however, one piston drives the internal pressure
9 upstream to the test section and the other piston drives the external chamber pressure. The
10 pressure shaft allows for independent control of the pulsatile pressure. The attachment points
11 of the circular cam that couples the two drive shafts can be adjusted to provide the phase
12 (between 0 and 360 degrees) between the motions of the two shafts. This phase difference
13 provides simulation of a wide range of SPAs, including the disease prone coronary arteries
14 (approximately 250 degrees). Since the flow is related to wall shear stress (WSS) and the
15 pressure is related to the circumferential strain (CS), the pulsatile WSS and pulsatile CS are
16 independent and uncoupled.

17
18 The present invention is a system for hemodynamic simulation comprises a
19 vessel having properties of a blood vessel, a reservoir containing a quantity of fluid, tubing
20 connecting the vessel and reservoir, and at least one pump for circulating the fluid within the
21 system. Fluid can be tissue culture medium or blood analog fluid, and the vessel may include
22 mammalian cells attached to its inside. A drive system, comprising two reciprocating drive
23 shafts that are coupled by a cam, enables the uncoupling of pulsatile flow and pulsatile
24 pressure to provide independent control over wall shear stress and circumferential strain.
25 The shaft drives two pumps that are 180 degrees out-of-phase and are connected upstream
26 and downstream of the vessel, and effect this uncoupling.

27

28

29 BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING.

30

31 Fig. 1A is a top plan schematic view of the hemodynamics simulator of the
32 present invention.

33

34 Fig. 1B is a side view illustrating the 4-bar linkage of the present invention.

35

36 Fig. 1C is a more detailed schematic diagram of the embodiment of Fig. 1A.

1 Fig. 1D is a schematic diagram of an embodiment which includes a bypass of
2 the compliant vessel.

4 Fig. 2 is a plot of the diameter (circles) and pressure (triangles) waveforms as
5 a function of time with a zero degree stress phase angle (SPA) difference.

7 Fig. 3 is a plot of the diameter (triangles), pressure (crosses) and flow (squares)
8 waveforms as a function of time with a sixty degree stress phase angle (SPA) difference.

10 Fig. 4 is a plot of the diameter (squares), pressure (triangles) and flow
11 (diamonds) waveforms as a function of time with a ninety degree stress phase angle (SPA)
12 difference.

14 Fig. 5 is a plot of the diameter (squares), pressure (triangles) and flow
15 (diamonds) waveforms as a function of time with a one hundred eighty degree stress phase
16 angle (SPA) difference.

17 Fig. 6 illustrates the structure of the support and support mount.

20 Fig. 7 illustrates the shape of the support rod.

22 Fig. 8 illustrates fluid flow through the support rod and vessel using different
23 shaped support rods. The arrow in Panels A and B represents the direction of fluid flow:

24 Panel A: using a linear shaped support rod;

25 Panel B: using a tapered support rod.

27 Fig. 9 illustrates another embodiment of the noise filter (vibration damper).
28 Panels A and B represent two different configurations.

30 Fig. 10 is a schematic diagram of a second embodiment of the present invention.

33 DETAILED DESCRIPTION OF THE INVENTION.

35 The present invention is a hemodynamic simulator **10**, shown schematically in
36 Figure 1A, and in greater detail in Fig. 1B. The hemodynamic simulator **10** comprises a

1 sample chamber **12** (which will also be referred to herein as "compliant vessel") which may
2 comprise either a non-rigid tube that contains mammalian cells, a blood vessel excised from
3 a mammal, or other biocompatible substrate containing cells or onto which cells can be grown
4 or attached thereto. Sample chamber **12** is connected to a reservoir **14** containing an
5 appropriate fluid **16**, which may comprise a tissue culture medium, blood or a blood analog
6 fluid, physiological saline solution (generally a solution of 0.9% sodium chloride ("NaCl")), as
7 known to those skilled in the art), or other buffered solution.

8 Reservoir **14** generally is a sterilizable container comprising a plurality of
9 fittings **20** which function to provide, for example only and not intended as any limitation
10 except as described in the claims, temperature probe insertion; pH probe insertion; inflow and
11 outflow of culture medium **16**; inflow and outflow of one or more gases, such as, but not
12 limited to, CO₂, nitrogen, oxygen, air or other gas or gaseous mixture, such as 5% CO₂ in air;
13 as may be required; media sampling port; addition of acid, base or other buffering agent for
14 the adjustment or other control of medium pH. Reservoir **14** is generally made of a standard
15 laboratory grade glass, but, as known to those skilled in the art, may also comprise any type
16 of sterilizable plastic vessel which can meet the system's requirements.

17
18 The system **10** includes a first pump **22**, which is generally used to provide a
19 steady flow of fluid **16** through the system, such that fluid **16** flows between reservoir **14** and
20 compliant vessel **12** through tubing **24**. In one embodiment of the present invention, the flow
21 rate is maintained as a steady rate, controlled by first pump **22**. In this embodiment, first
22 pump **22** is a centrifugal pump, such as one the Biomedicus 520d (manufactured by
23 Biomedicus Corp., Minneapolis, MN). In another embodiment of the present invention, first
24 pump **22** is a peristaltic pump, such as that sold by MasterFlex Corp., New Brunswick
25 Scientific (New Brunswick, NJ) or other commercial laboratory supply manufacturers. Other
26 types of pumps can also be employed as first pump **22**, such as a DISC-FLO® pump, a gear
27 pump, or other pumps which must provide a constant volumetric flow.

28
29 In the embodiment wherein the first pump **22** is a peristaltic pump, a noise filter
30 **26** is required, in order to dampen the noise (high frequency vibrations) created by the
31 movements of the peristaltic pump (Fig. 1B). The noise filter may also be referred to herein
32 as a pulse damper, and is commercially available from laboratory supply houses, such as the
33 PULSE DAMPENER® (Cole-Parmer Corp., Vernon Hills, IL). The noise filter **26** also serves
34 as a bubble trap, preventing the passage of bubbles that may be generated by the pump. As
35 will be described in further detail below, the system may also include a bypass to prevent
36 bubbles from entering the compliant vessel (see Fig. 1C).

1 An alternate embodiment of the noise filter **26** is illustrated in Fig. 9, the
2 differences between the noise filter in Figs. 9A and 9B being the configuration of the
3 container **72**. Container **72** comprises a inlet **74** and outlet **76** ports for the inflow and
4 outflow of fluid **16** from the system, respectively. Air inlet **78** and outlet **80** ports are also
5 fitted into the container. In addition, a pressure relief valve (not shown) can be fitted into
6 container **72**.

7 The alternate embodiments of the noise filter reduce the amount of fluid required by the
8 system, compared to the amount of fluid used when the commercial noise filter is employed.

9
10 Generally, it is preferred to utilize a minimal amount of fluid **16** in order to
11 reduce the costs of media utilization, drug treatment, and cell by-product (such as, but not
12 limited to, proteins, metabolites and like) detection and the like. In the embodiment shown
13 in Figs. 1A-1C, approximately 100 ml of fluid are employed. The length of the tubing from the
14 vibration damper **26** to the upstream connector also provides additional high frequency steady
15 flow pump induced vibration damping.

16
17 Tubing **24** generally comprises any suitable type of laboratory tubing which is
18 capable of being sterilized. Such tubing includes that sold under the trademark of Tygon®
19 (Norton Co., Worcester, MA); PharMed® tubing (Trademark of PharMed Group Corporation,
20 Miami, FL), silicone tubing, or other comparable laboratory or medical-surgical tubing from
21 other manufacturers.

22
23 The length of the upstream tubing is chosen so as to minimize the total volume
24 of fluid used in the system. Its length is calculated to provide a maximum flow rate, and to
25 avoid turbulence in the system, based upon boundary layer theory, as known to those skilled
26 in the art, and described further below.

27
28 The compliant vessel **12** is supported proximate its ends **28**, **30** by a pair of
29 supports **32** which are held in place by a pair of rigid mounts **34**, respectively. The mounts
30 **34** and supports **32** preferably are as shown in Figs. 6-8, each mount including an opening **62**
31 therethrough, to accommodate a support **32** therein. To facilitate the alignment of the
32 compliant vessel **12** within the support system, a support rod **64** is inserted into aperture **66**
33 located on each support mount **62**. A set screw **68** may be used to retain the support rod **64**
34 in position. The support mount **34** preferably is made from a non-corrosive, durable material,
35 and capable of withstanding autoclaving; stainless steel is one such material. Each support
36 **32** comprises a tube having ends **70** shaped to fit the ends **28**, **30** of compliant vessel **12**

1 (Figs. 8A and 8B). As shown in Fig. 8B, the tapered end **70** of support **32** provides a fit at the
2 ends of compliant vessel **12** such that there is a negligible disturbance of fluid flow, in
3 contrast to the disturbance that would occur if the end of support was linear (Fig. 8A). The
4 ends of the compliant vessel **12** are attached to each support using clamps, suturing, or other
5 methods known to those skilled in the art. In one embodiment of the present invention, the
6 supports **32** are manufactured from TEFLO[®] (polytetrafluoroethylene, DuPont Co.,
7 Wilmington, DE) or stainless steel, but other suitable, biocompatible materials can be
8 substituted.

9
10 Depending upon the which properties (WSS, CS, pressure) are to be studied,
11 the compliant vessel **12** may be surrounded by an external chamber **36**, but external chamber
12 is not required under all circumstances. In such instances, the external chamber is opened
13 to the atmosphere. External chamber **36** is a sealed chamber that has a port with which the
14 chamber can be filled with a fluid such as water or other fluid, and a second port through
15 which contents of the chamber **36** can be pressurized by connection to one of the pumps **42**.
16 External chamber **36** may also be a jacketed chamber, enabling a cooled or heated fluid to
17 circulate around the compliant vessel **12** in order to maintain the temperature required by the
18 contents of the compliant vessel **12**, and the chamber connected to a circulating bath, such
19 as those manufactured by the Neslab Corporation.
20

21 Although not essential to the operation of the hemodynamic simulator **10** of the
22 present invention, an additional length of tubing **24** can be added to function as a compliant
23 vessel bypass **38** (Fig. 1C). The bypass tubing **38** is connected both upstream and
24 downstream of the compliant vessel **12**, so that if problems occur when the system is started
25 from a zero flow rate and pressure to the desired flow and pressure, such as bubble formation,
26 the bypass can be used until proper conditions are achieved, at which point the bypass **38** is
27 closed off or removed, and flow is resumed through the compliant vessel **12**.

28
29 The support **32** is made from tubing having an inner diameter (I.D.) that
30 matches the I.D. of both the compliant vessel **12** and the upstream tubing. By having the I.D.
31 of the support matching the I.D. of the vessel and tubing, this prevents flow separation and
32 an underdeveloped flow regime from occurring. The wall of the support **32** should taper to the
33 outside such that the compliant vessel **12**'s I.D. does not bend abruptly as it is placed over the
34 support. This provides a flush I.D. surface between the support **32** and the compliant vessel
35 **12** and greatly minimizes flow separation. One possible configuration is to have the upstream
36 tubing, the support **32** and the compliant vessel **12** to be made of one piece with a rigid

1 structure around the upstream end and support.

2

3 Drive System.

4

5 The system further comprises a plurality of pumps **40** and **42**, further
6 designated as second pumps **40** (also referred to herein as P1 and P2), and third pumps **42**
7 (also referred to herein as P3 and P4), respectively (Figs. 1A and 1B). As shown in Fig. 1A,
8 pumps P1 and P3 are connected to the "upstream" flow of the hemodynamic system **10** of the
9 present invention, pump P2 is connected to the "downstream" flow, and pump P4 is
10 connected to the external chamber **36**, providing external pressure on the compliant vessel
11 **12** contained therein. Fluid **16** or the like flows downstream back into reservoir **14**, in a
13 closed flow system; the culture fluid is recycled to conserve culture fluid, but if the culture
14 fluid becomes unsuitable for growth, such as caused by acid build-up therein, reservoir **14** can
15 be replaced with one containing a fresh quantity of fluid **16**, as appropriate. The various
16 components of the present invention are connected by sterile fittings, and components can
be changed, aseptically, as experimental or other conditions so require.

17

18 Each of pumps **40** and **42** is under the control of a drive system unit **44**, which
19 comprises a plurality of independent linear actuators **46**. These actuators **46** can be
20 individual, stand alone units, or may be controlled by one or more computer systems **48**. In
21 the embodiment in Fig. 1A, the second pumps **40** are connected by a shaft **50**, and the third
22 pumps **42** are connected by a second shaft **52**. In one embodiment of the present invention,
23 in which a 4-bar linkage mechanism is the drive system, a cam **54** affects the control of the
24 various second pumps **40** and third pumps **42**. In one embodiment of the present invention
25 (Fig. 1B) the drive system unit **44** comprises six computer-controlled linear actuators.

26 The hemodynamic simulator **10** includes a plurality of sensors **18** for measuring
27 hemodynamic parameters. These sensors **18** include a flow sensor, which may be placed
28 either upstream and/or downstream of the compliant vessel **12**. Such a flow sensor can be
29 an ultrasound Doppler probe, as known to those skilled in the art. The Doppler probe,
30 depending upon its position in the system, can either be a sterile probe, and/or a probe that
31 may or may not be fluid-contacting. An electromagnetic probe may also be used as a flow
32 sensor. In one embodiment of the present invention, the flow sensor is an ultrasonic
33 flowmeter (Transonics Systems, Inc.) which is positioned in-line and just upstream of the
34 compliant vessel. Flow rate variation over the length of the compliant vessel has been
35 negligible.

36

1 A pressure sensor **18** is used for monitoring the internal system pressure, and
2 positioned either upstream and/or downstream of the compliant vessel **12**. Pressure sensor
3 **18** can also be a blood pressure catheter (such as, for example, and not intended as a
4 limitation, a MILLAR® catheter (MPC-500 with pressure meter TCB500; Registered
5 Trademark of Millar Instruments Corp., Houston TX), in either a fluid contacting or non-
6 contacting version. Pressure sensor **18** may also be a pressure probe, such as those known
7 to those skilled in the art. In one embodiment of the present invention, the pressure sensor
8 is a catheter tip transducer (Millar) which is inserted upstream into the lumen of the
9 compliant vessel. Where cells are being used in the compliant vessel **12**, the pressure sensor
10 **18** is kept upstream to avoid damaging the cells. Pressure drop across the compliant vessel
11 has been shown to be negligible.

12
13 The linear actuators **46** may be selected from among those that comprise a cam
14 mechanism; a multi-bar linkage mechanism, such as an actuator comprising a four-bar
15 mechanism; a solenoid; a stepper motor; an electric motor, whether operated by alternating
16 current ("AC") or direct current ("DC"); a linear ball actuator; a belt driven actuator; a chain
17 driven actuator; or any other drive unit which is capable of producing a variable cyclic motion,
18 or any combination of the above actuators, such as, for example only, and not intended to be
19 a limitation, the combination of a cam mechanism and a 4-bar linkage mechanism and a DC
20 motor. The cyclic motion generated by the drive system unit can resemble that of a blood
21 pressure waveform in its magnitude, frequency and other properties, as known to those skilled
22 in the art. By adjustment of the drive system components, as known to those skilled in the art,
23 the extent of the phase differences among the second pumps **38** (P1-P4) can be adjusted, from
24 anywhere between 0 degrees and 360 degrees.

25
26 It has been classically known to those skilled in the art that pressure and flow
27 are coupled, and could not be uncoupled. Using the dynamic sinusoidal environment created
28 by the hemodynamics simulator **10** of the present invention, flow and pressure can be
29 uncoupled.

30
31 This uncoupling is achieved using the drive system **44** of the present invention,
32 comprising two reciprocating drive shafts **50** and **52** that are coupled via a circular cam **54**
33 (Fig. 1A). Each flow shaft **50** or **52** drives two piston pumps P1 and P2, or P3 and P4,
34 respectively (at opposite ends) that are 180 degrees out-of-phase and are connected to the
35 recirculating flow loop upstream and downstream of the compliant vessel **12** (test section).
36 The flow shaft allows independent control of pulsatile flow with no pulsatile circumferential

1 strain. The second (pressure) shaft 52 also drives two piston pumps that are 180 degrees out-
2 of-phase; however, one piston drives the internal pressure upstream to the compliant vessel
3 12 (test section) and the other piston drives the external chamber pressure. The pressure
4 shaft allows for independent control of the pulsatile pressure. The attachment points of the
5 circular cam 54 that couples the two drive shafts can be adjusted to provide the phase
6 (between 0 and 360 degrees) between the motions of the two shafts. This phase difference
7 provides simulation of a wide range of SPAs, including the disease prone coronary arteries
8 (approximately 250 degrees). Since the flow is related to wall shear stress (WSS) and the
9 pressure is related to the circumferential strain (CS), the pulsatile WSS and pulsatile CS are
10 independent and uncoupled. In this process, changes in the upstream pressure may have an
11 effect on the downstream pressure, such that if the stroke of the upstream pumped is
12 changed, the stroke of the downstream pump does require compensation.

13
14 Prior to setting up the hemodynamic simulator 10 of the present invention,
15 system components are sterilized. Sterilization can be effected, depending upon the
16 components of the system, by methods such as autoclaving, ethylene oxide (EtO) treatment,
17 ultraviolet light irradiation, gamma irradiation, and other methods known to those skilled in
18 the art.

19
20 The hemodynamic simulator 10 is generally run at a temperature of
21 approximately 37 degrees Centigrade, but it can be operated at temperatures ranging from
22 approximately 20 degrees Centigrade to approximately 50 degrees Centigrade. As shown
23 in Fig. 1B, the "test section", representing the compliant vessel 12, and support means 32 and
24 34 can be immersed in a water bath 56 of the appropriate temperature. The hemodynamic
25 simulator 10 can be operated for a duration ranging from as short as a few minutes, for
26 example, 5-10 minutes, to more extended lengths of time, such as, between approximately 72
27 hours to 168 hours. In a preferred situation, the hemodynamic simulator is operated over a
28 period of between approximately 5 hours and approximately 72 hours. A limiting factor in the
29 duration of the hemodynamic simulator 10's operation is maintenance of sterility of the
30 system.

31
32 It is to be understood that factors such as the geometry of the vessel, the
33 diameter of the vessel, the viscosity of the medium used, the pressure, and the flow rate of
34 the medium through the vessel, are among the factors that determine the wall shear stress
35 (WSS), and that when reference is made to WSS, these factors are taken into consideration.

1 By insertion of the compliant vessel **12** within the external chamber **36**, the
2 effects of diameter variation, caused by circumferential strain and wall shear stress, can be
3 studied, in the absence of pulsatile pressure (condition 2).

4 The diameter variation of the compliant vessel is measured using a diameter
5 sensor. The diameter sensor can be a non-contacting ultrasound transducer **82** (such as a
6 single element transducer V312 10/.25 and pulser-receiver unit 5072, both from Panametrics
7 Co., Waltham, MA, not shown). The ultrasound probe position must be perpendicular to and
8 aligned with the center of the diameter of the test specimen in order to sense the diameter.
9 One beam passes through the specimen (a pulse), differences in material densities results in
10 peaks and beam profile alterations that are detected with the receiver, and are subsequently
11 acquired and processed using a computer which includes an oscilloscope with peak detection
12 software and appropriate analytical software. A linear cross-sectional profile of the specimen
13 is then detected, providing the dimensions of the outer and inner walls, and consequently, wall
14 thickness. The probe can be positioned anywhere in the test section to provide dimensions.
15 Absolute and relative dimensions can be obtained, for example, relative dimensions are
16 sufficient for monitoring diameter variations. The dimensions are monitored and acquired,
17 via the computer, in real-time along with pressure, flow and other measurements. A multi-
18 array ultrasound probe can also be used to monitor diameter variation. The diameter sensor
19 can also utilize lasers, video imaging, magnetic resonance imaging, other imaging modalities,
20 or can be a contacting probe, such as known to those skilled in the art.

21
22 All data signals are acquired by the computer system, which is not shown in the
23 drawings. The ultrasound diameter monitoring requires a peak detection algorithm. Phase
24 angle is determined using Fast Fourier Transforms ("FFT"). Some signals are used for
25 monitoring, and feedback control such as mean pressure, is monitored and adjusted via a
26 motor controlled downstream reactor.

27
28 The wall shear stress waveform is determined based on the measured flow
29 waveform and the mean diameter according to Womersley (1955, and incorporated herein by
30 reference).

31
32 Initially, the flow is run at a low flow rate, and then the flow is adjusted to a high
33 flow rate. The resistor **58** is adjusted to provide a mean pressure, and the oscillatory drive
34 system unit **44** is engaged to oscillate the ends of the sample, depending upon the
35 experimental conditions under investigation, by varying the movement of second pumps **40**,
36 (P1 and P2) and third pumps **42** (P3 and P4). The resistor **58** is a device that controls the

1 degree of occlusion of the downstream flow to achieve a desired mean pressure. Examples
2 of resistors suitable for use in the present invention include a gear motor controlled clamp
3 device that controls occlusion of the downstream tubing; valves, pinch clamps or other types
4 of laboratory clamps.

5
6 The hemodynamic simulator **10** of the present invention can simulate the
7 important features of the mammalian hemodynamic environment,

8
9 The first hemodynamic conditions to be discussed are the fluid flow, pressure,
10 and diameter variation (circumferential strain). The fluid flow and pressure (and consequently
11 diameter variation) can be manipulated to allow for precise control of the cyclic pulsatile fluid
12 flow and pressure magnitude and phase. The fluid flow and pressure, and consequently, the
13 diameter variation in the case of tubular geometry, can be manipulated to allow for precise
14 control of the cyclic pulsatile fluid flow and pressure magnitude and phase. A "tubular
15 geometry case", as used herein, is intended to refer to the use of curved vessels (for example,
16 half a toroid), bifurcated vessels (including variation such as branched, Y-shaped, T-shaped,
17 and the like). In other instances, the vessels employed are linear and non-branched.

18
19 There are several possible system configurations available, depending upon the
20 simulation conditions.

21
22 Complete control of the fluid flow and pressure relations attainable are:

23
24 Condition 1-fluid flow and pressure magnitude and phase
25 (0-180 degrees) [i.e., wall shear stress 10 dynes per square
26 centimeter +/- 10 dynes per square centimeter and 8% diameter
27 variation with their phase variation (angle) at 180 degrees for a
28 compliant vessel **12** made of silicone;

29
30 Condition 2-pulsatile flow and no pulsatile pressure
31 (diameter variation), magnitude and phase;

32
33 Condition 3-pulsatile pressure (diameter variation) and no
34 pulsatile flow magnitude and phase; and

35
36 Condition 4-pulsatile flow and pulsatile pressure (no

1 diameter variation) magnitude and phase.

2
3 In a compliant vessel where the transmural flux (hydraulic conductivity and/or
4 permeability) can be monitored, conditions 1 and 2 require no change or considerations.
5 Condition 3 requires consideration of the potential transmural reflux due to active transmural
6 pressure modulation. Condition 4 requires consideration of potential external pressure
7 augmentation due to increased hydraulic conductivity and/or permeability that can be
8 compensated for via an external pressure feedback control mechanism.

9
10 Under Condition 1, the following combinations of second pumps **40** (P1 and P2),
11 and third pumps **42** (P3 and P4) can be utilized: a) all four pumps, P1, P2, P3 and P4; b) P1,
12 P2 and P4; or c) P1 and P3; or d) P2 and P4.

13
14 Under Condition 2, second control pumps **40**, P1 and P2 are utilized.

15
16 Under Condition 3, third pumps **42**, P3 and P4 are utilized.

17
18 Under Condition 4, second pumps **40** (P1 and P2) and third pumps **42** (P3 and
19 P4) are utilized.

20
21 The conditions are chosen according to the desired hemodynamic environment
22 under simulation. Condition 1 is the most physiologically prevalent condition. The upstream,
23 downstream, and external pressures are modulated, primarily, with respect to amplitude,
24 phase, and frequency to achieve the desired hemodynamic environment. These parameters
25 are effected using the controls of the drive system unit, a laboratory computer system **48**.

26
27 The system thus operates with one of the second pumps **40** (in this instance,
28 pump P1) affecting the upstream portion of the compliant vessel **12**, and exerting its actions
29 in a "pushing" manner along the compliant vessel **12**. A similar action is obtained with the
30 third pump **42** (pump P3) acting on the upstream end of compliant vessel. In contrast, the
31 other of the second pumps **40** (in this instance, pump P3) affects the downstream portion of
32 the compliant vessel **12**. Third pump P4 exerts an external pressure on the compliant vessel
33 **12**. The different actions of the pumps affect the movement/pulsation of the compliant vessel
34 **12**.

35
36 The effects of wall shear stress (WSS) are studied when the upstream second

1 pump P1 and the downstream third pump P3 are engaged. In this situation, these pumps are
2 working against each other by being 180 degrees out of phase, and the upstream pump P1
3 causes an increase in the flow rate, while the downstream pump P3 causes a decrease in flow
4 rate, resulting in no external pressure, and a combination of shear stress and pulsatile fluid
5 flow through the compliant vessel **12**.

6
7 When the hemodynamic simulator **10** of the present invention is used for
8 studying the effects of circumferential strain (CS) on the compliant vessel **12**, one second
9 pump, P1 and third pump P4, are used. In this situation, the first pump **22** (the steady flow
10 pump) can be shut off, and second pump P1 provides the upstream pressure, while third pump
11 P4 provides the external pressure on the compliant vessel **12**.

12
13 The novel part of the apparatus is the drive system which induces the sinusoidal
14 flow component and the diameter variation. In one embodiment of the present invention, the
15 drive system **44** is a 4-bar linkage mechanism, shown schematically (Fig. 1). The second
16 pumps **40** (P1 and P2) are connected by a first linkage **102**. Third pumps **42** (P3 and P4) are
17 connected by a second linkage **104**. Each linkage connects to piston **106** of each pump. The
18 linkages are connected to cams **54** by shafts **50** and **52**, and each cam **54** is connected at **108**
19 to a DC motor **110**. Each drive shaft **52**, **54**, is connected by an adjustable pivot **112**, which
20 adjusts the length of the stroke of each pumps' piston **106**. The drive system comprises two
21 reciprocating drive shafts which are coupled through a circular cam. The phase between the
22 motion of the two shafts can be varied by adjusting the angle between the attachment points
23 of the two shafts on the common cam **54** (for example, zero degrees for in-phase, 180 degrees
24 for out-of-phase). One of the shafts **50** drives two piston pumps which are 180 degrees out-of-
25 phase and are connected to the recirculating flow loop upstream and downstream of the
26 compliant vessel **12**. The second shaft **52** drives two piston pumps which are also 180 degrees
27 out-of-phase; one pump feeds the flow loop upstream of the compliant vessel, the second
28 pump drives the external chamber. The two out-of-phase piston pumps driving the internal
29 flow loop act in a push-pull fashion. When the external chamber **36** is open to the atmosphere
30 (when the second drive shaft **52** is disconnected) and the stroke volumes of the push-pull
31 pumps on the first drive shaft are equal, a sinusoidal flow is generated, but with negligible
32 pressure variation because of the push-pull action. When the system is run in this fashion
33 (second shaft disconnected) it is possible to ave sinusoidal flow (superimposed on the steady
34 flow) with negligible pressure or diameter variation. To induce diameter variation, the second
35 shaft is connected at any desired phase relative to the first shaft by adjustment of the cam **54**.
36 When both piston pumps on this shaft are interfaced to this system, it is possible to adjust

1 their stroke volumes so that the pressure in the external chamber and in the elastic compliant
2 vessel are nearly constant (as a result of the push-pull action), and there is diameter variation
3 driven by the volume change between the elastic compliant vessel and the external chamber
4 (one fills while the other empties). When the system is run in this fashion, there is sinusoidal
5 flow with defined diameter variation and phase angle relative to flow, but there is negligible
6 pressure variation. This enables the present invention to uncouple pressure and stretch.
7

8 To introduce pressure variation in phase with diameter variation, which is
9 considered to be the most physiological condition, the drive line to the external chamber is
10 disconnected, and the chamber is left open to the atmosphere. In this mode of operation, both
11 pressure and diameter variation are driven by the upstream piston pump P3 on the second
12 shaft **50**. Some interaction occurs between the pumps driven on the two shafts, but the
13 volume flows driven by the second shaft **50** (controlling diameter variation) are very small
14 compared to those driven by the first shaft **52** (which controls flow), and they can be adjusted
15 nearly independently.

16 The present invention was designed to overcome the current technological
17 limitations in vascular research by physically simulating the normal and diseased physiologic
18 states. The present invention achieves a precise and complete physiologic environment by
19 uncoupling the major hemodynamic forces, WSS and CS, thereby permitting independent
20 control over the magnitude and phase of the pulsatile WSS and CS to achieve a wide range
21 of SPA. The present invention experimentally simulates real hemodynamic patterns, both
22 simple and complex patterns, while maintaining sterility of the system, and employing a
23 minimal volume of media demanded by cell and tissue culture systems.
24

25 The advantage of cell and tissue culture systems is that the tools of cell and
26 molecular biology are easily employed. This integrative approach to the design of the present
27 invention resulted in a system that is quick and easy to assemble and disassemble while
28 maintaining the cell culture integrity that is important for biological assays. The test chamber
29 of the present invention facilitates the insertion and removal of the test specimens. The test
30 specimens are generally endothelial cell coated silicone elastic tubes which are placed in the
31 hemodynamic simulator of the present invention, and yield biological results relevant to the
32 normal and diseased cardiovascular system.
33

34 Those skilled in the art have classically considered it well known that pressure
35 and flow are coupled. However in the dynamic sinusoidal environment, established by the
36

1 present invention, flow and pressure can be uncoupled, thereby providing independent control
2 over WSS and CS.

3
4 The present invention not only provides a means for studying hemodynamics
5 in normal and diseased states, but it also can be used in tissue engineering, to test or train the
6 function of bypass vessels prior to their use in coronary bypass surgery, or to investigate
7 cryopreserved vessels for research or medical use. Current coronary bypass surgery most
8 often utilizes vessels from the hemodynamically unstrenuous saphenous vein (in the lower leg)
9 as the bypass vessel. The present invention can be used to train the vessel to the strenuous
10 hemodynamic environment of the coronary arteries. As can be seen from the foregoing, these
11 applications are ultimately related to the treatment of cardiovascular disease.
12

13
14 The present invention may also be useful for analysis of bone mechanics, and
15 effects of flow and related parameters on the development of osteocytes, chondrocytes and
16 the like. Shear stress is known to increase the production of types II and I collagen, and other
17 extracellular products, thus potentiating the fact that further mechanical stimuli, such as
18 strain and shear stress, would further improve production of extracellular products. Stem
19 cells can be stimulated to differentiate by mechanical stimuli, such as shear stress, strain, or
20 solute transport systems. Other applications include, but are not intended to be limited to,
21 effects on cell and tissue culture, tissue engineering, effects in complex artery geometries,
22 effects on cardiac valves and their *in vitro* evaluation, evaluation and standardization of
23 imagery diagnostic methods using vascular phantoms, effects of pharmacological agents on
24 cells and tissues, materials testing in standard environments and in microgravity
environments, and on cells co-cultured in a mixed bioreactor.

25
26 Example 1. Preparation of silicone tubing for attachment and growth of endothelial cells.
27

28 In this example, the vessel chosen for growth of endothelial cells is a silastic
29 tubing, sold by Dow-Corning, Midland, MI under the brand name of SYLGARD 184®
30 elastomer, or Silastic (MDX4-4210), Medical Grade tubing, and used to prepare elastic artery
31 models. These models were prepared using the method described by Lee and Tarbell (1997,
32 and hereby incorporated by reference), and included the preparation of models of human
33 linear and bifurcating arteries.

34
35 For the preparation of linear elastic vessels, a pair of symmetric, half-cylindrical
36 grooved molds made of a plastic, such as PLEXIGLASS, are machined to have a diameter that

1 matches the inner diameter of the elastic model described above. In one preferred
2 embodiment, the linear elastic vessels have a length of approximately 29 centimeters and an
3 inner diameter of approximately 0.79 centimeters, in another embodiment of the present
4 invention, vessels having a length of approximately 15 cm are employed. A solid wax,
5 cylindrical core is prepared by distributing melted wax (CARBOWAX®, Union Carbide Co.)
6 into the mold, and placing the mold inside another cylindrical mold of the same plastic; in the
7 preferred embodiment, this second mold has a diameter of approximately 0.95 centimeters,
8 so as to produce an annular layer having a diameter of approximately 0.080 centimeters. A
9 solution of SYLGARD 184® and a curing agent, prepared in accordance to methods known
10 to those skilled in the art, is poured into this part of the mold, vacuum deaerated by methods
11 known to those skilled in the art, and then cured. After curing, the elastic vessel is removed
12 from the mold.

13
14 The elastic vessels are treated to promote cell attachment before being
15 inoculated with cells. Briefly, the vessels are hydrophylized in a 70% sulfuric acid solution,
16 boiled in distilled water and then sterilized by autoclaving. The vessels are then coated with
17 a layer of fibronectin (30 micrograms/ml in Modified Eagle's Medium ("MEM")), a tissue
18 culture medium known to those skilled in the art, fibronectin is obtained from commercial
19 sources).

20 While vessels having inner diameters ranging from between 1-10 mm can be
21 used, vessels having an inner diameter of approximately 8 mm (0.79) cm has been shown to
22 be an optimal inner diameter, and allow for the use of multiple tubes in the present invention
23 while keeping the overall size of the present invention, and the consumption of cell culture
24 media and other expendables, within a range that is manipulable by laboratory personnel. In
25 the system shown in Figs. 1A-1C, approximately 100 ml of fluid are employed. Each end of
26 the vessel is inserted into position in the present invention as has been previously described,
27 using the supports 32 and mounts 34. Where necessary, sterile tubing connectors are also
28 employed to enable tubing and other components to be connected into the system under
29 aseptic conditions.

30
31 Example 2. Tissue culture conditions.

32
33 Endothelial cells ("ECs") were obtained either from bovine aortas ("BAECs"), or
34 from human umbilical veins ("HUEVCs"), and cultured by growth as primary cultures, using
35 procedures described in Sill et al. (1995). the contents of which is hereby incorporated by
36 reference.

1 The BAECs were the cells most commonly used with the present invention. An
2 inoculum of between 60,000-80,000 cells per square centimeter is used twice, once to enable
3 the cells to adhere to the surface of the vessel for a 45 minute time period, and a second time
4 after rotating the position of the vessel 180 degrees to enable the vessel's other side to
5 become coated. The cells are grown in a monolayer until confluence is achieved, in a 37
6 degree centrifugal tissue culture incubator in an atmosphere of 5% CO₂ in air. The preferred
7 growth medium **16** is Dulbecco's Modified Eagle's Medium ("DMEM", obtained commercially
8 from Sigma Chemical Corp., St. Louis, MO), containing 10% Fetal Bovine Serum ("FBS",
9 obtained commercially), 1% L-glutamine and 1% antibiotics (penicillin-streptomycin solution.
10 For experiments, the medium comprised DMEM without FBS, and 1% bovine serum albumen
11 ("BSA") and 1% antibiotics (penicillin-streptomycin solution; BSA and the antibiotics are
12 commercially available from Sigma Chemical Corp.). MEM (also obtained from Sigma) may
13 be employed, depending upon the type of cells being utilized. Generally, the pH of the culture
14 fluid is maintained at approximately pH 7.2, +/- 0.05, but a pH in the range between
15 approximately 7.0 to approximately 7.5 is acceptable.

16
17 Requirements of the fluid **16** include having a viscosity that can be elevated to
18 achieve conditions of physiologic stress at modest flow rates. Dextran is used within the fluid
19 while the present invention uses vessels of approximately 0.79 cm diameter; in instances
20 employing vessels of smaller diameter, addition of dextran is not necessary. The fluid should
21 be free of Phenol Red and serum so as not to interfere with measurements of other cellular
22 products, such as prostacycline or nitric oxide.

23
24 In addition to the use of tissue culture media, other physiological fluids, such
25 as blood from a mammal such as sheep, cow, pig, rabbit, or human cord blood or human blood,
26 can be utilized. Artificial or analog blood fluids can also be used. Among the blood analog
27 fluids known to those skilled in the art is an admixture of glycerol in water, and adjusted to
28 have a viscosity comparable to blood.

29
30 Example 3. Effect of different stress phase angles: zero degree SPA.

31
32 Fig. 2 is a plot of the diameter (circles) and pressure (triangles) waveforms as
33 a function of time with a zero degree stress phase angle (SPA) difference.

34
35 Changes in the diameter of the compliant vessel **12** can be measured by one of
36 several methods known to those skilled in the art. These include the use of such non-

1 contacting methods as ultrasound or laser light, or the use of an elastic strain gauge, which
2 is in physical contact with the specimen (the compliant vessel). In the present invention, the
3 preferred method of monitoring the changes in compliant vessel diameter is with an
4 ultrasound transducer (Panametrics Co., not shown) which is mounted through the exterior
5 chamber wall and which is focused on the compliant vessel.

6

7 The computer controlled drive unit 44 is capable of generating different
8 waveforms, which can range from a sine wave, as employed in this and the subsequent
9 examples (Figs.2-6), or which can be a blood pressure waveform, such as a known waveform
10 taken from a reference text, or determined experimentally on a human. For convenience in
11 establishing the parameters of the present invention, sine waves were chosen. The flow
12 waveform represents the rate of flow of the culture medium 16 or other fluid through the
13 system as a function of time. The flow rates, in milliliters per minute, have been normalized
14 so as to fit on a scale ranging from plus 1 to minus 1. Similarly, data representing the
15 pressure on the compliant vessel 12, expressed in mm of mercury, and the degree of
16 distortion of the diameter of the compliant vessel (diameter waveform) have also been so
17 normalized.

18

19 The rate of wall shear in the compliant vessel was measured using a
20 photochromic method of flow visualization for use in elastic tubes. Using a focused laser
21 beam having a specific wavelength, the laser beam passes through the vessel, containing a
22 photo-sensitive dye of a corresponding wavelength, and causes the dye to change color and
23 generate a dye line within the fluid flow. Using a video camera to record the displacement
24 of the dye line caused by the pulsating laser beam, the near wall velocity profile form which
25 the wall shear rate can be determined from the slope at the wall, using methods described in
26 Rhee and Tarbell (1994, and incorporated by reference herein). In this example, the preferred
27 laser is a nitrogen laser with a wavelength in the range of the ultraviolet (VSL337ND, from
28 Laser Science Inc.).

29

30 A polyalkylene glycol ether, described in Weston *et al.* (1996, and incorporated
31 by reference herein) would be usable because this agent has the rheological properties
32 comparable to blood, and the photodynamic properties that are compatible with the material
33 from which the compliant vessels were manufactured.

34

35 Fig. 2 illustrates that when there is no difference in the phase angle between
36 the flow and the pressure, the pressure waveform and the diameter waveform are similar to

1 each other.

2

3 Example 4. Effect of different stress phase angles: sixty degree SPA.

4

5 Fig. 3 is a plot of the diameter (triangles), pressure (crosses) and flow (squares)

6 waveforms as a function of time with a sixty degree stress phase angle (SPA) difference.

7

8 When the phase angle between the flow and the pressure are sixty degrees out

9 of phase, the pressure waveform and the diameter waveform remain similar to each other,

10 while the flow waveform is shifted (Fig. 3).

11

12 Example 5. Effect of different stress phase angles: ninety degree SPA.

13

14 Fig. 4 is a plot of the diameter (squares), pressure (triangles) and flow

15 (diamonds) waveforms as a function of time with a ninety degree stress phase angle (SPA)

16 difference.

17

18 When the phase angle between the flow and the pressure are ninety degrees

19 out of phase, the pressure waveform and the diameter waveform remain similar to each other,

20 while the flow waveform is shifted (Fig. 4).

21

22 Example 6. Effect of different stress phase angles: one hundred eighty degree SPA.

23

24 Fig. 5 is a plot of the diameter (squares), pressure (triangles) and flow

25 (diamonds) waveforms as a function of time with a one hundred eighty degree stress phase

26 angle (SPA) difference.

27

28 When the phase angle between the flow and the pressure are one hundred

29 eighty degrees out of phase, the pressure waveform and the diameter waveform remain

30 similar to each other, but the flow waveform is shifted to an even greater extent compared to

31 when they are either 60, or 90 ninety degrees out of phase (compare Fig. 5 with Figs. 2-4).

32

33 Example 7. Compliant vessels.

34

35 Example 1 described the use of vessel models, modeled after the structure of

36 actual human aortic vessels. In addition to using models of vessels, other vessels can be used

1 in conjunction with the present invention. These can be chosen from the group consisting of
2 an artery, an artificial artery, a vein, human umbilical tissue, or a non-rigid tube. The artery
3 may comprise a bovine aorta, or a human coronary artery. The vein may comprise bovine
4 veins, or human veins such as a human leg vein or a human umbilical vein. Bovine tissue can
5 be obtained from commercial supply sources, such as Vec Technologies, Ithaca NY and human
6 umbilical materials can be obtained a local hospital, or a commercial sources such as
7 Clonetics, Vec Technologies, or other sources known to those skilled in the art. In addition
8 to studying the effects of hemodynamic conditions on endothelial cells, other types of cells can
9 also be used, including smooth muscle cells, cartilage cells, osteocytes, embryonic and adult
10 stem cells, and the like.

11
12 The tubing employed as the vessel can have any geometry, ranging from
13 geometries, such as, for example only and not intended as any limitation, straight, curved,
14 bifurcating, branched or the like. The vessel may also be chosen from any chamber, whether
15 having a parallel flow, a radial flow, etc. The vessel may also be made of any material, such
16 as, but not limited to, materials such as silicone, collagen, an artery, a vein, glass, tissue
17 culture grade plastics or the like; such materials are considered to be biocompliant. The
18 compliant vessel can thus have any combination of these properties.

19
20 Example 8. An embodiment for studying hemodynamics on multiple vessels.

21
22 In this embodiment of the present invention (shown schematically in Fig. 10,
23 and in which like reference numerals refer to like elements), the hemodynamics simulator **200**
24 can be used to study hemodynamic properties of a plurality of compliant vessels **12**. This
25 embodiment is similar to that described in Figs. 1A and 1B, but comprises a plurality of
26 compliant vessels **12**, a plurality of reservoirs **14**, a first pump **22** which has been adapted to
27 pump fluid through a plurality of tubing **24**, and a plurality of noise filters **26**, as needed, as
28 has been described for that embodiment (Fig. 1B). The compliant vessels **12** are enclosed in
29 a plurality of external chambers **36**. Under such conditions, compliant vessels **12** can be
30 studied with and/or without an external chamber **34** under otherwise comparable
31 experimental conditions. The drive system unit **44** is similar to that described previously
32 (Figs. 1A-1B). Although a plurality of reservoirs **14** are illustrated in Fig. 10, a single reservoir
33 could be used to supply all of the compliant vessels **12**, or multiple reservoirs containing
34 different types of culture media or other biological fluid **16**, could be used, for examining the
35 effects of either different cell types under identical stress conditions, or the effects of
36 different fluids on a cell line, or other combinations desired to be examined by one skilled in

1 the art.

2
3 Therefore, although this invention has been described with a certain degree of
4 particularity, it is to be understood that the present disclosure has been made only by way of
5 illustration and that numerous changes in the details of construction and arrangement of parts
6 may be resorted to without departing from the spirit and scope of the invention.
7
8

9 References

10
11 Berthiaume, F., Frangos, J.A. 1993. "Flow effects on endothelial cell signal transduction,
12 function and mediator release." Flow-dependent regulation of vascular function. Bevan et al.,
13 Oxford Univ. Press, New York.

14
15 Carosi, C.G., Eskin, S.G., and McIntire, L., 1992. Cyclic strain effects on production of
16 vasoactive materials in cultured endothelial cells. *J. Cellular Physiol.* 151:29-36.

17
18 Lee, C.S., and Tarbell, J.M. 1997. Wall shear rate distribution in an abdominal aortic
19 bifurcation model: Effects of vessel compliance and phase angle between pressure and flow
20 waveforms. *J. Biomech. Engr.* 119:333-342.

21
22 Rhee, K., and Tarbell, J.M. 1994. A study of the wall shear rate distribution near the end-to-
23 end anastomosis of a rigid graft and a compliant artery. *J. Biomechanics* 27:329-338.

24
25 Qiu, Y.C., and Tarbell, J.M. 2000. Interaction between wall shear stress and circumferential
26 strain affects endothelial cell biochemical production. *J. Vascular Res.* 37:147-157.

27
28 Seliktar, D., Nerem, R.M. et al. 2000. Dynamic mechanical conditioning of collagen gel blood
29 vessel constructs induces remodeling in vitro. *Ann. Biomedical Eng.* 28:351-362.

30
31 Sampio, B.E., and Widmann, M.D. 1990. Enhanced production of endothelial-derived
32 contracting factor by endothelial cells subjected to pulsatile stretch. *Surgery* 108:277-282.

33
34 Weston, M.W., Rhee, K., and Tarbell, J.M. 1996. Compliance and diameter mismatch affect
35 the wall shear rate distribution near an end-to-end anastomosis. *J. Biomechanics* 29:187-198.

1 Womersley, J.R. 1955. Method for the calculation of velocity, rate of flow and viscous drag
2 in arteries when the pressure gradient is known. J. Physiol. 127:553-563.

3
4 All patents and references cited herein are hereby incorporated by reference
5 in their entirety.